

FINAL REPORT

ROLE OF CORTICOSTEROIDS IN BONE LOSS DURING SPACE FLIGHT\*

PRINCIPAL INVESTIGATOR: THOMAS J. WRONSKI, PH.D.  
DEPARTMENT OF PHYSIOLOGICAL SCIENCES  
UNIVERSITY OF FLORIDA  
GAINESVILLE, FL 32610

CO-INVESTIGATORS: BERNARD P. HALLORAN, PH.D.  
DEPARTMENT OF MEDICINE  
UNIVERSITY OF CALIFORNIA  
SAN FRANCISCO, CA 94121

SCOTT C. MILLER, PH.D.  
RADIOBIOLOGY DIVISION  
UNIVERSITY OF UTAH  
SALT LAKE CITY, UT 84112

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## OBJECTIVES

The primary objective of this research project is to test the hypothesis that corticosteroids contribute to the adverse skeletal effects of space flight. To achieve this objective, serum corticosteroids, which are known to increase during space flight, must be maintained at normal physiologic levels in flight rats by a combination of adrenalectomy and corticosteroid supplementation via implanted hormone pellets. Bone analyses in these animals will then be compared to those of intact flight rats that, based on past experience, will undergo corticosteroid excess and bone loss during space flight. The results will reveal whether maintaining serum corticosteroids at physiologic levels in flight rats affects the skeletal abnormalities that normally develop during space flight. A positive response to this question would indicate that the bone loss and decreased bone formation associated with space flight are mediated, at least in part, by corticosteroid excess.

## BACKGROUND

Space flight is known to induce alterations in calcium homeostasis. Gemini, Apollo, and Skylab astronauts exhibited hypercalciuria and negative calcium balances (1-3). Since the skeleton is the major reservoir of calcium in the body, increases in the urinary excretion of calcium presumably reflect bone loss. Photon absorptiometry revealed that the bone mineral density of the calcaneus declined by approximately 4% in Skylab crewmembers after 84 days of orbital flight (4). More recently, bone ultrasound and quantitative computed tomography detected losses of from 4 to 13% of primarily cancellous bone in the calcaneus and tibia of a cosmonaut stationed on MIR for 6 months (5). Although it is commonly assumed that the observed bone loss is due primarily to increased bone resorption, recent biochemical data indicate that decreased bone formation may also be involved (5).

Bone histomorphometric analyses are necessary to define more completely the skeletal effects of space flight. Unfortunately, such analyses are not feasible in astronauts due to the traumatic nature of the bone biopsy procedure. For this reason, bone histologic studies in experimental animals subjected to space flight are of considerable interest. Rats placed in orbit aboard Soviet Cosmos biosatellites and the space shuttle exhibited an inhibition of periosteal bone formation (6-8) and loss of cancellous bone in the long bone metaphysis (9-12). Calcium kinetic analyses (13) and measurements of the number of osteoclasts (9-

12), the bone resorbing cells, indicate that bone resorption was not increased in rats during space flight. On the other hand, the number of osteoblasts, the bone forming cells, was found to be decreased in rats subjected to space flight (9-12). Therefore, the observed loss of cancellous bone in flight rats appears to be due primarily to an inhibition of bone formation rather than a stimulation of bone resorption.

It is commonly assumed that the adverse skeletal effects of space flight are due to loss of mechanical loading in a weightless environment. Nevertheless, some lines of evidence suggest that other factors may be involved. If the bone changes induced by space flight are due solely to mechanical unloading, these changes should be confined to weightbearing bones. However, skeletal abnormalities have been detected in bones of flight rats that lack a weightbearing function such as the maxilla, mandible, and calvarium (14-16). These findings may be interpreted as evidence that the skeletal effects of space flight are systemic rather than confined to weightbearing bones. The failure of on-board centrifugation to prevent the inhibition of periosteal bone formation in flight rats (17) also indicates that microgravity is not solely responsible for bone loss during space flight.

Endocrine factors in general and corticosteroids in particular may be involved in the etiology of the apparently systemic skeletal effects of space flight. In support of this concept, plasma cortisol was found to be significantly increased in Skylab astronauts for the duration of their long-term missions (1). Several investigators have reported that adrenal hypertrophy occurred in rats placed in orbit aboard Cosmos biosatellites (2,3). Exogenous administration of corticosteroids induces marked hypercalciuria (18) and skeletal alterations that are similar to those observed during space flight, including cancellous bone loss (19-21), decreased numbers of osteoblasts (22-24), and an inhibition of periosteal bone formation (24,25).

In summary, the above findings suggest that the changes in calcium homeostasis and bone associated with space flight may be mediated, at least in part, through the action of corticosteroids. This hypothesis has not been adequately tested to date. The current research project is designed to manipulate serum corticosteroids by a combination of adrenalectomy and exogenous supplementation with implanted hormone pellets. Maintenance of equivalent physiologic levels of serum corticosteroids in flight and ground-based rats with subsequent bone analyses will determine whether corticosteroid excess is essential for the development of skeletal abnormalities during space flight.

## METHODS

The experimental animals were male Sprague Dawley rats that were 6 weeks of age and weighed an average of 165g at launch. All rats were anesthetized with an IM injection of ketamine hydrochloride (50 mg/kg body weight) and xylazine (10 mg/kg body weight) and subjected to bilateral adrenalectomy or sham surgery at 4 days prior to launch. At the time of surgery, pellets composed of cholesterol with dissolved corticosterone and aldosterone were implanted in each adrenalectomized (ADX) rat. The proper doses of the hormones to achieve normal circulating levels of corticosterone and aldosterone were established in prior supporting ground-based studies. Each sham-operated rat was implanted with a placebo cholesterol pellet. On the day before launch, all rats were injected SC with calcein at a dose of 15 mg/kg body weight to label bone forming surfaces. Shortly afterwards, six ADX flight rats and six sham flight rats were loaded in each of two animal enclosure modules (AEM) and transported to the space shuttle Columbia for launch on 6/20/96 (STS-78). On the day of launch, baseline ADX and sham rats were sacrificed for collection of serum and bone samples. Other ADX and sham rats were placed in ground-based AEMs or standard vivarium (VIV) cages. The experiment therefore consisted of the following 8 groups of rats (N=6/group):

- |                  |             |
|------------------|-------------|
| 1. Baseline ADX  | 5. AEM ADX  |
| 2. Baseline Sham | 6. AEM Sham |
| 3. Flight ADX    | 7. VIV ADX  |
| 4. Flight Sham   | 8. VIV Sham |

After a 17 day space flight, the ADX and sham flight rats were necropsied between 4 and 6 hours after landing. Serum samples were collected and stored at -80°C until their corticosterone and aldosterone concentrations were measured by radioimmunoassay techniques. The adrenal glands in sham rats were carefully dissected free of adjacent tissues and weighed with a Mettler balance. Various bones including both tibiae, femora, lumbar vertebrae, and caudal vertebrae were stripped of musculature. The left tibia was frozen for subsequent measurements of bone dry and ash weights. Other bones were placed in 10% phosphate-buffered formalin for 24 hours for tissue fixation. The bone samples were then dehydrated in increasing concentrations of ethanol and embedded undecalcified in methyl methacrylate. For cancellous bone analyses, longitudinal sections were cut at a thickness of 4  $\mu$ m with an AO Autocut/Jung 1150 microtome. These sections were stained according to the Von Kossa method with a tetrachrome

counterstain for measurements of cancellous bone volume (%), osteoclast surface (%), an index of cancellous bone resorption, and osteoblast surface (%), an index of cancellous bone formation. All data were collected in cancellous bone tissue at distances greater than 1 mm from the growth plate-metaphyseal junction to exclude the primary spongiosa.

The distal half of the right tibia was dehydrated in ethanol and acetone, then embedded undecalcified in a styrene monomer that polymerizes into a polyester resin (Tap Plastics, San Jose, CA). The tibial diaphysis 1-2 mm proximal to the tibiofibular junction was then sawed into 50-75  $\mu$ m thick cross sections with a Buhler Isomet low speed saw. Cortical bone area, cortical width, and marrow area were measured in these cross sections. Other measurements include the area of newly formed bone between the calcein label and the periosteal surface as well as the distance between the calcein label and the periosteal surface at 100  $\mu$ m intervals around the periphery of cortical bone. This distance was divided by the time interval between administration of the calcein label and landing (18 days) to calculate periosteal mineral apposition rate. Similarly, the area of newly formed cortical bone along the periosteal surface was divided by the same time interval to calculate the periosteal bone formation rate.

All histomorphometric measurements were performed with the Bioquant Bone Morphometry System (R&M Biometrics Corp., Nashville, TN). Data are expressed as the mean for each group  $\pm$  SD. Statistical differences among groups were evaluated by ANOVA followed by Fisher's Protected Least Significant Difference (PLSD) test for multiple comparisons. P values less than 0.05 were considered to be significant.

## RESULTS

All rats gained substantial body weight during the course of the experiment (Figure 1). The ADX and sham flight rats exhibited at least as much weight gain as the ground-based AEM and VIV rats. In fact, the mean body weight of the ADX flight group was slightly but significantly increased compared to the mean body weight of all other groups. These findings indicate that space flight was well tolerated by the flight rats.

Mean values for adrenal gland weights for the 4 sham groups are shown in Figure 2. The sham flight group had significantly increased adrenal gland weights compared to baseline and ground-based AEM and

VIV sham groups. This finding is consistent with adrenal hypertrophy and corticosteroid excess in sham flight rats.

Figures 3 and 4 depict mean values for serum corticosterone and aldosterone, respectively. Sham flight rats exhibited a significantly higher mean value for serum corticosterone by at least a factor of 2 compared to all other groups. This finding undoubtedly reflects a stress response to re-entry and postflight handling in these animals. The ground-based AEM and VIV sham groups also exhibited at least a strong trend for increased serum corticosterone compared to all 3 ADX groups. In contrast, the mean value for serum corticosterone remained at 50-60 ng/ml in all ADX groups, which is equivalent to normal physiologic levels of the hormone in rats. Similarly, mean serum aldosterone (Figure 4) was maintained at the normal physiologic level of approximately 100 pg/ml in the 3 ADX groups. Therefore, the implanted hormone pellets were found to successfully deliver normal levels of corticosterone and aldosterone to the systemic circulation of ADX rats.

The dry and ash weights of the left tibia (data not shown) were significantly greater in the flight, AEM, and VIV groups than the baseline groups due to growth of the former groups during the experimental period. However, no significant differences in tibial dry and ash weights were detected among the flight groups (ADX and sham) and the ground-based AEM and VIV groups (ADX and sham).

Data for cancellous bone volume in the proximal tibial metaphysis are shown in Figure 5. The mean values were nearly the same for all 8 groups of rats with no significant differences among them. ADX and sham flight rats also did not exhibit even a trend for decreased cancellous bone volume in the lumbar vertebra, caudal vertebra, and femoral neck (data not shown). Similarly, osteoclast surface (Figure 6), an index of bone resorption, and osteoblast surface (Figure 7), an index of bone formation, varied little in the proximal tibial metaphysis of all groups. These cellular variables also did not differ in the lumbar and caudal vertebrae of flight rats (ADX and sham) compared to the ground-based AEM and VIV rats (data not shown).

Structural data for cortical bone in the tibial diaphysis are shown in Figures 8-10. A growth-related increase in cortical bone area and width was detected in the older flight, AEM, and VIV groups compared to the younger baseline groups. However, when comparing rats of the same age, these variables did not differ in the flight groups (ADX and sham)

compared to the AEM and VIV groups. Marrow area was nearly the same in all 8 groups of rats.

Age-related decreases in periosteal bone formation rate (Figure 11) and periosteal mineral apposition rate (Figure 12) occurred in the flight, AEM, and VIV groups compared to the baseline groups. However, the mean values for these variables were very similar in the flight groups (ADX and sham) compared to the ground-based AEM and VIV groups.

## CONCLUSIONS

All preflight procedures were accomplished as planned. The rats were successfully adrenalectomized (ADX) and the implanted hormone pellets delivered physiologic levels of corticosterone and aldosterone to the systemic circulation. The substantial increase in body weight that occurred in all rats indicated that the ADX/supplemented rats were healthy and that the flight rats tolerated space flight well. The observed adrenal hypertrophy in the intact sham flight rats was also a positive finding in that it was suggestive of corticosteroid excess in these animals.

Unfortunately, the experimental objective, which was to test the hypothesis that corticosteroids contribute to bone loss during space flight, could not be achieved due to lack of bone changes in intact flight rats. These animals exhibited normal cancellous bone mass at several different skeletal sites. Furthermore, both cancellous and cortical bone formation were found to be normal in flight rats compared to ground-based control rats. The results clearly indicate that space flight has minimal effects on bone mass and bone formation in rapidly growing rats. This finding is surprising in view of previous reports of cancellous bone loss (9-12) and an inhibition of bone formation (6-12) in rats subjected to space flight. However, it is important to note that the rats from most of these previous studies were older than the rats from the current study. In addition, the former rats were often housed singly while in space compared to the group housing for the animals of our experiment. Finally, the strain of the rats also varied among the different flight experiments. Therefore, the negative findings of the current study emphasize the importance of rat age, strain, and housing conditions for the development of bone changes during space flight. These factors are crucially important for the planning of future experiments involving use of rats as an animal model for the adverse skeletal effects of space flight.

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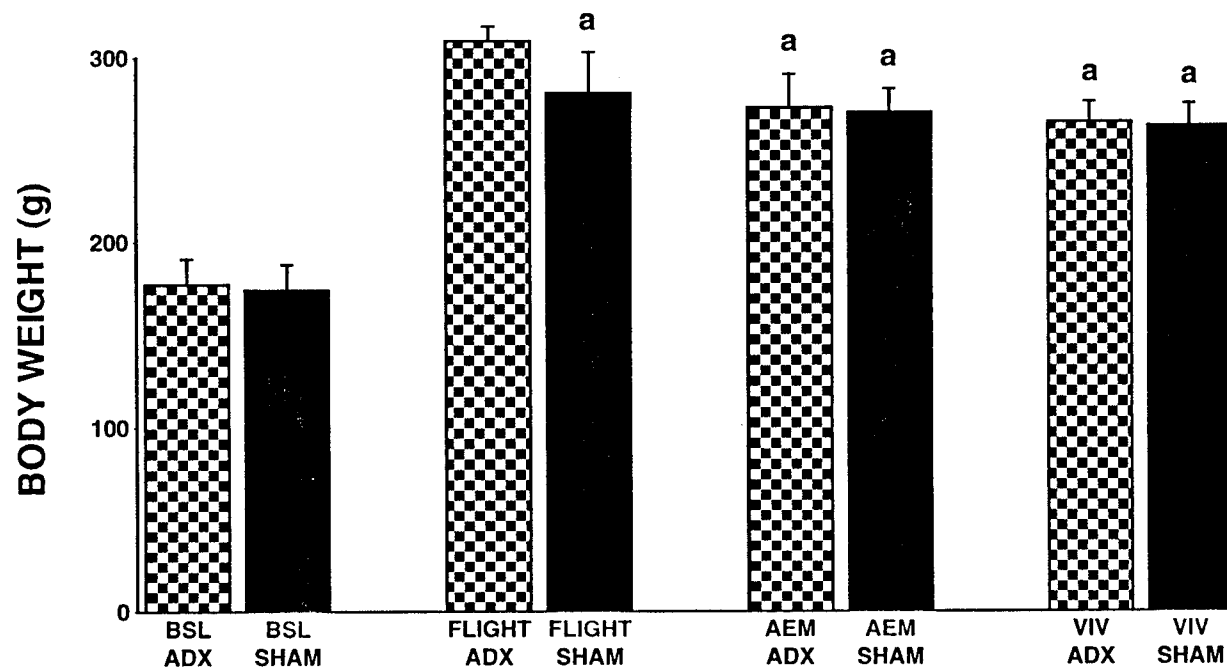
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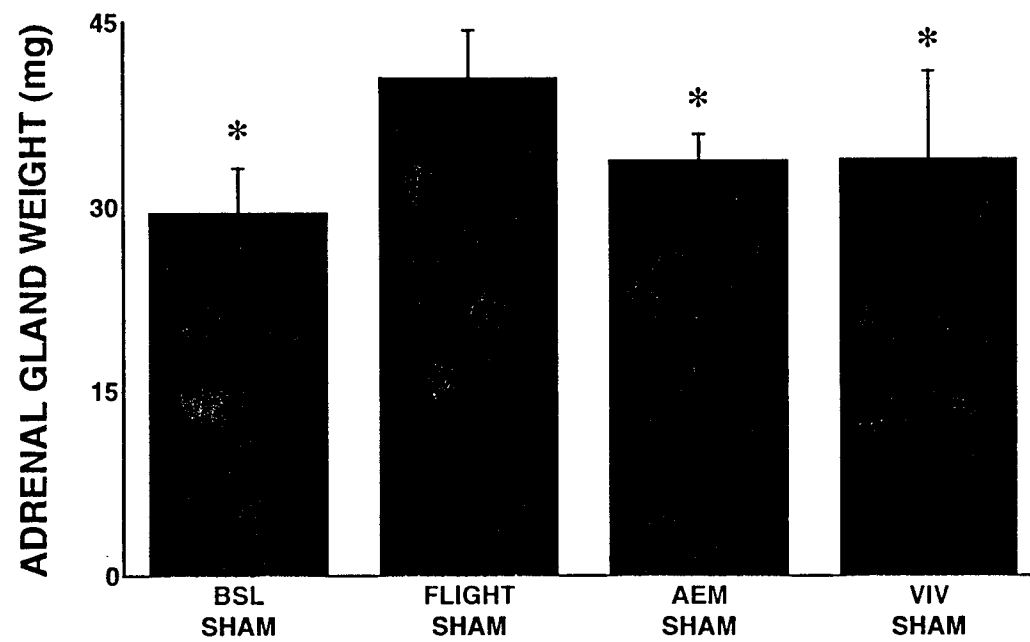
## NON-TECHNICAL SUMMARY

Corticosteroid hormones that are secreted by the adrenal glands in response to stressful situations are known to be increased during space flight. These hormones are also known to have adverse effects on bone. Therefore, the experiment was designed to determine whether excess secretion of corticosteroid hormones by the adrenal glands contributes to the bone loss associated with space flight. A certain group of flight rats had their adrenal glands removed surgically, but were then supplemented with normal levels of corticosteroids by implanted hormone pellets. Another group of flight rats with intact adrenal glands would presumably experience corticosteroid excess. A comparison of bone between these groups would reveal whether maintaining corticosteroids at normal levels in flight rats affects the bone changes that occur during space flight. Although the planned hormonal manipulations were successful, the experimental objective could not be achieved due to lack of the expected bone changes in flight rats with intact adrenal glands. These animals had normal amounts of bone mass and normal levels of bone formation compared to ground-based control rats. Therefore, space flight was found to have minimal effects on bone mass and bone formation in rapidly growing rats. This negative result may be a consequence of rapid bone growth in young rats, strain of rat, and/or group housing conditions during space flight. Therefore, the findings emphasize the importance of rat age, strain, and housing for the planning of future space flight experiments.

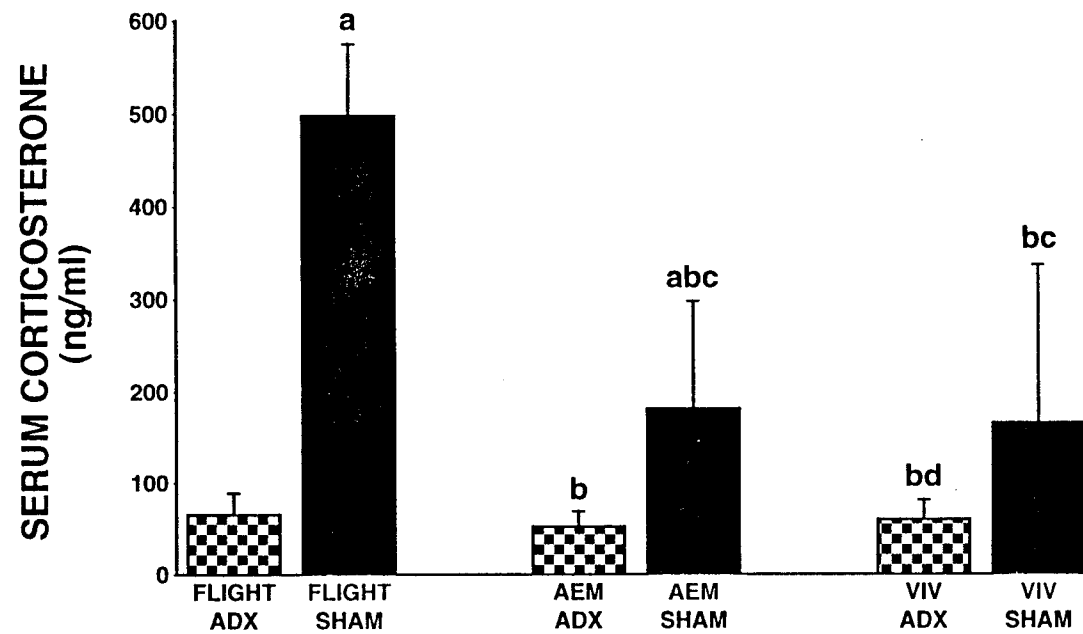


a: vs Flight ADX ( $p < 0.05$ )  
b: vs Flight Sham ( $p < 0.05$ )  
c: vs AEM ADX ( $p < 0.05$ )  
d: vs AEM Sham ( $p < 0.05$ )  
e: vs VIV ADX ( $p < 0.05$ )

**FIGURE 1**

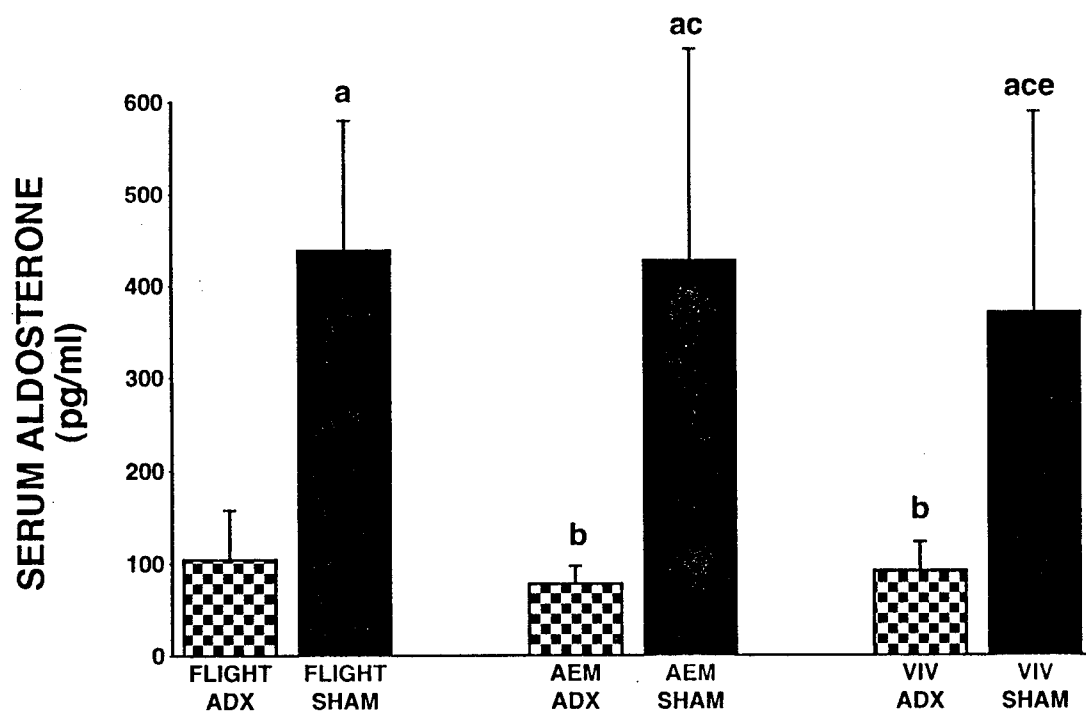


**FIGURE 2**



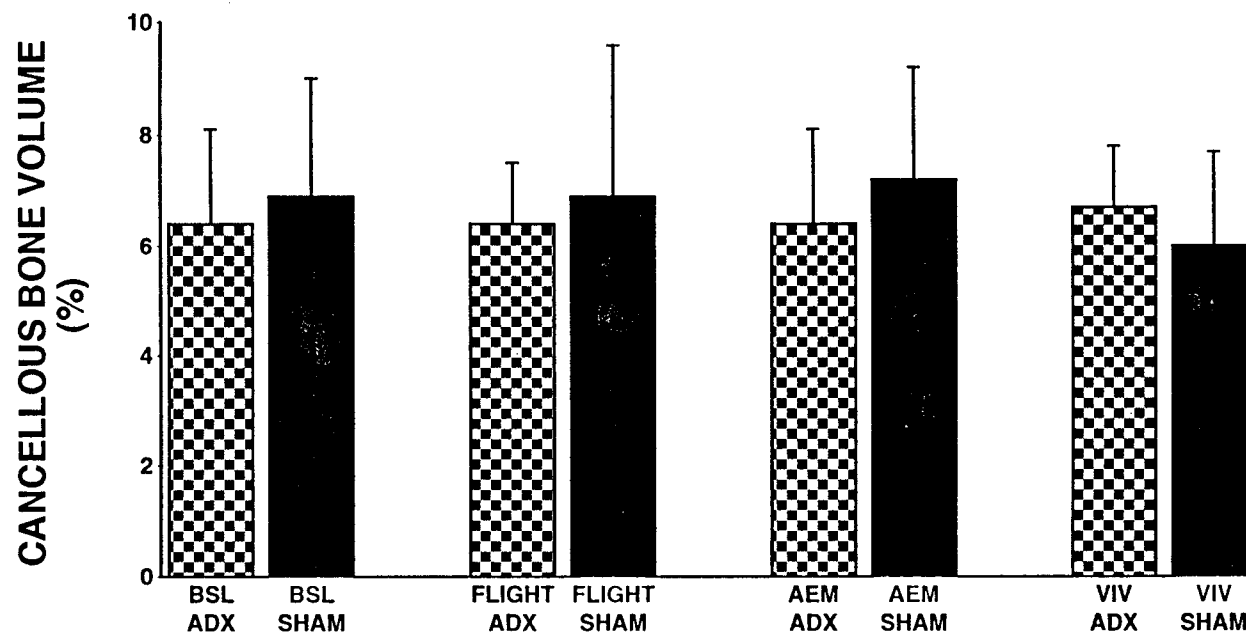
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b: vs Flight Sham ( $p < 0.05$ )  
c: vs AEM ADX ( $p < 0.05$ )  
d: vs AEM Sham ( $p < 0.05$ )  
e: vs VIV ADX ( $p < 0.05$ )

**FIGURE 3**



a: vs Flight ADX ( $p < 0.05$ )  
b: vs Flight Sham ( $p < 0.05$ )  
c: vs AEM ADX ( $p < 0.05$ )  
d: vs AEM Sham ( $p < 0.05$ )  
e: vs VIV ADX ( $p < 0.05$ )

**FIGURE 4**



a: vs Flight ADX ( $p < 0.05$ )

b: vs Flight Sham ( $p < 0.05$ )

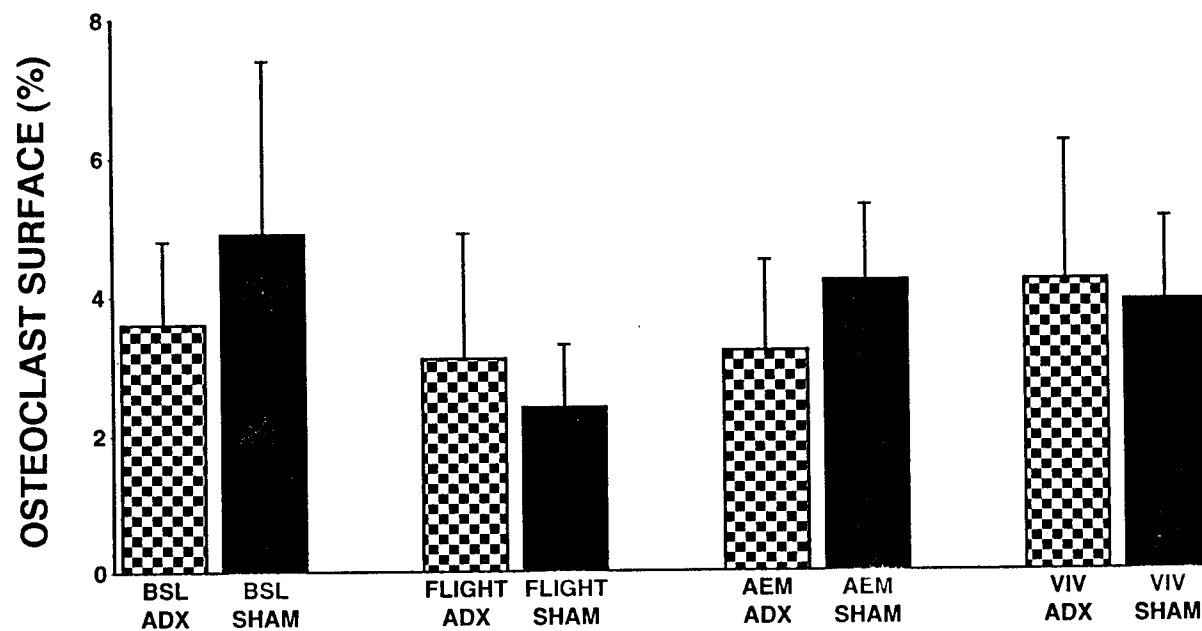
c: vs AEM ADX ( $p < 0.05$ )

d: vs AEM Sham ( $p < 0.05$ )

e: vs VIV ADX ( $p < 0.05$ )

**FIGURE 5**





a: vs Flight ADX ( $p < 0.05$ )

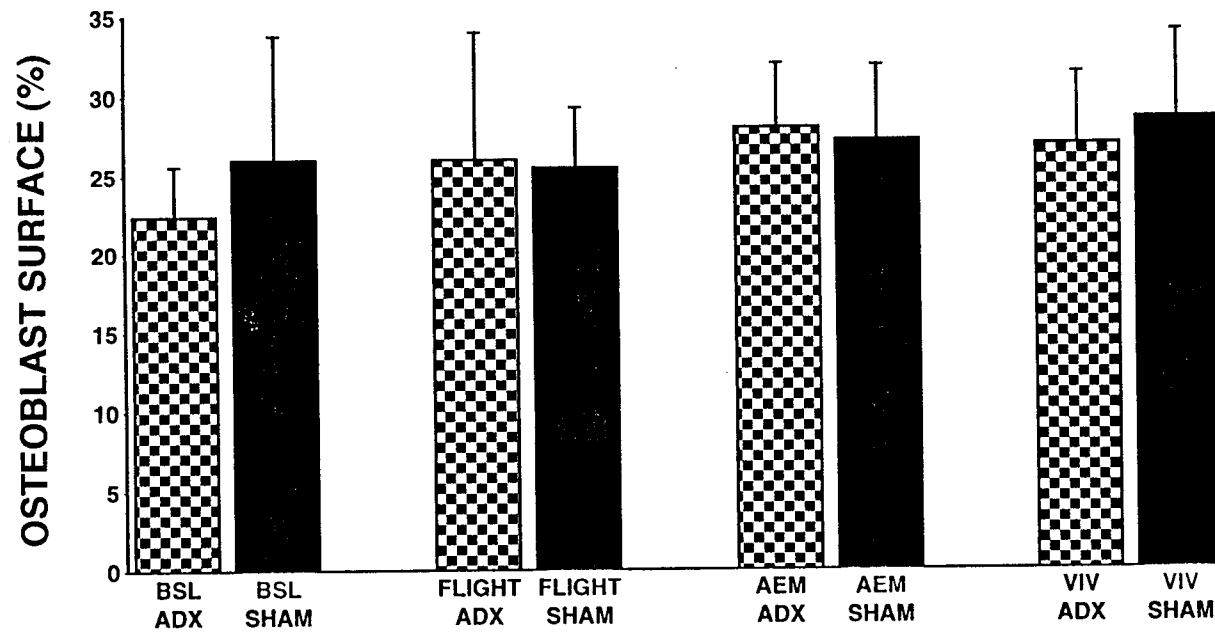
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c: vs AEM ADX ( $p < 0.05$ )

d: vs AEM Sham ( $p < 0.05$ )

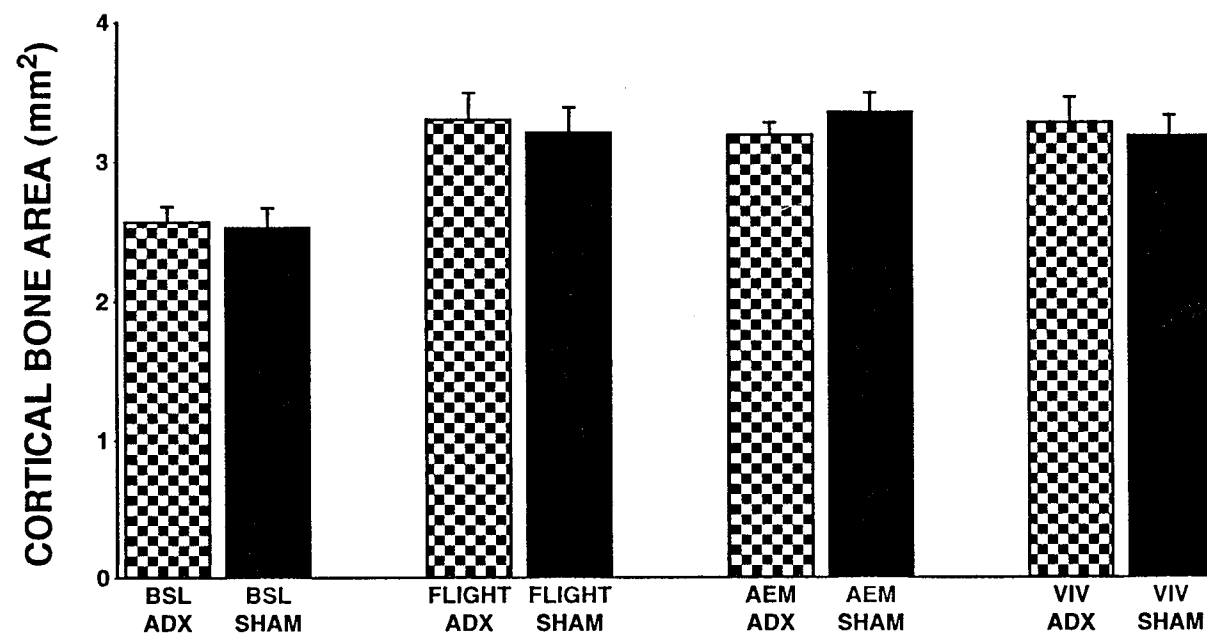
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**FIGURE 6**



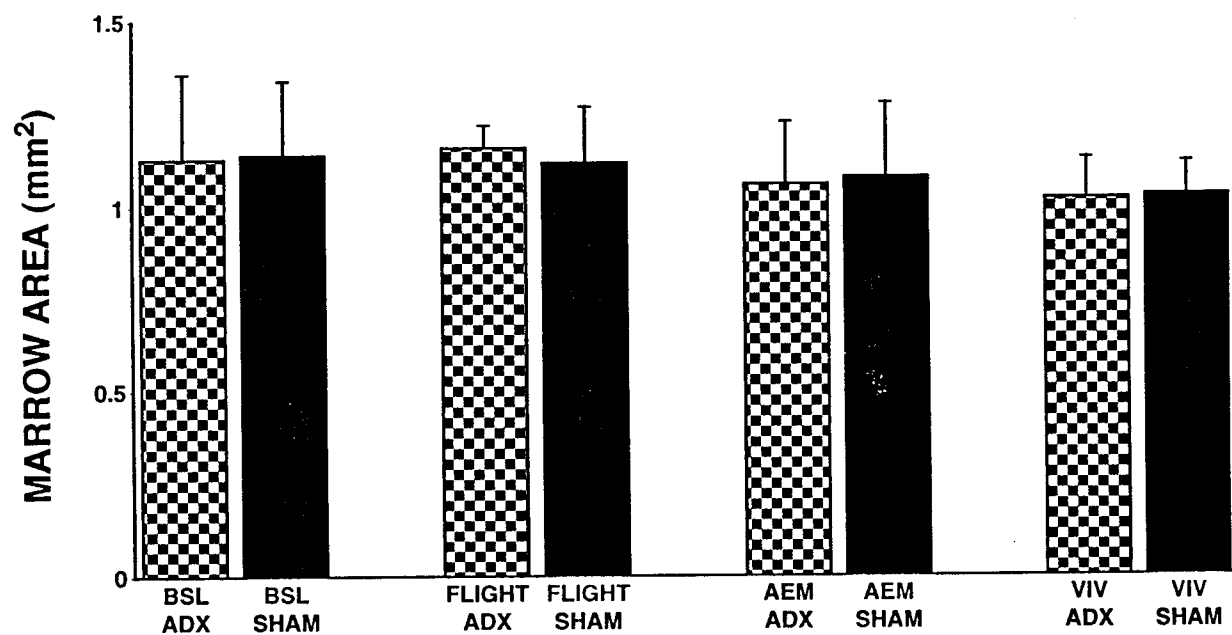
- a: vs Flight ADX ( $p < 0.05$ )
- b: vs Flight Sham ( $p < 0.05$ )
- c: vs AEM ADX ( $p < 0.05$ )
- d: vs AEM Sham ( $p < 0.05$ )
- e: vs VIV ADX ( $p < 0.05$ )

**FIGURE 7**



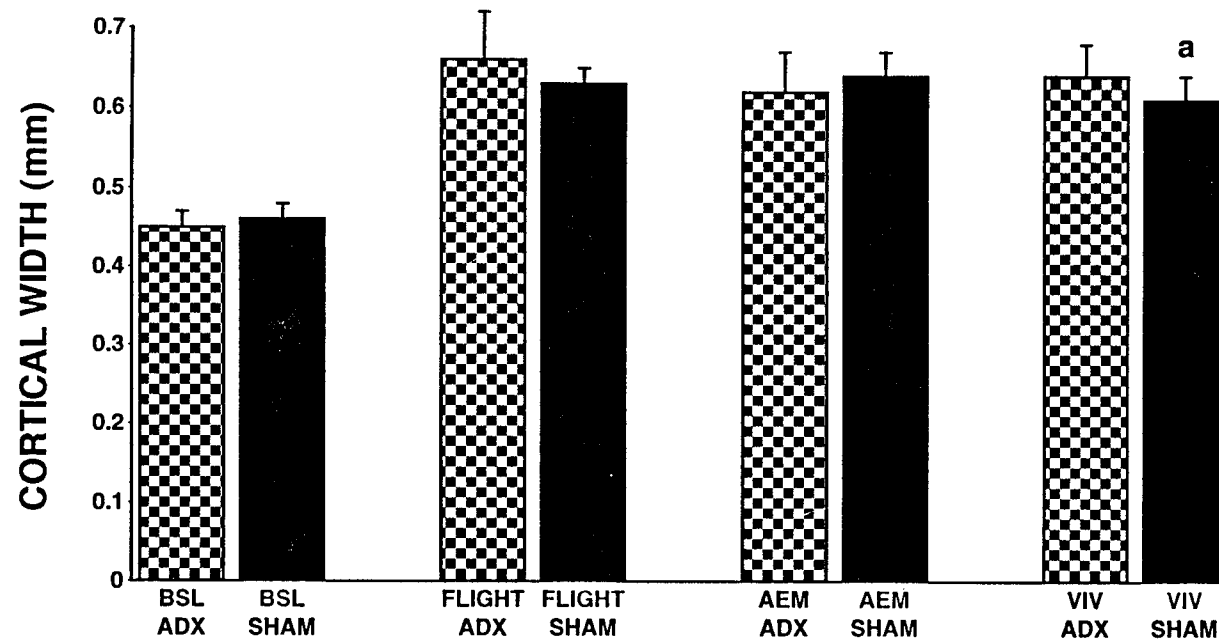
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- b: vs Flight Sham ( $p < 0.05$ )
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- e: vs VIV ADX ( $p < 0.05$ )

**FIGURE 8**



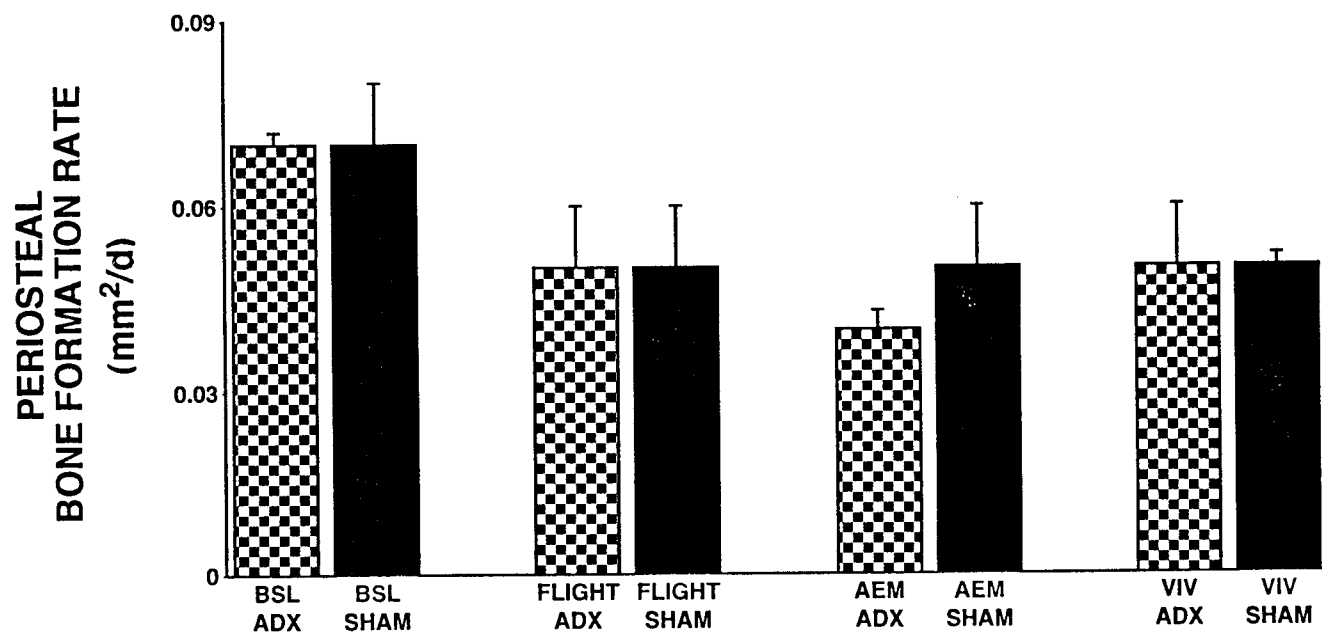
- a: vs Flight ADX ( $p < 0.05$ )
- b: vs Flight Sham ( $p < 0.05$ )
- c: vs AEM ADX ( $p < 0.05$ )
- d: vs AEM Sham ( $p < 0.05$ )
- e: vs VIV ADX ( $p < 0.05$ )

**FIGURE 9**



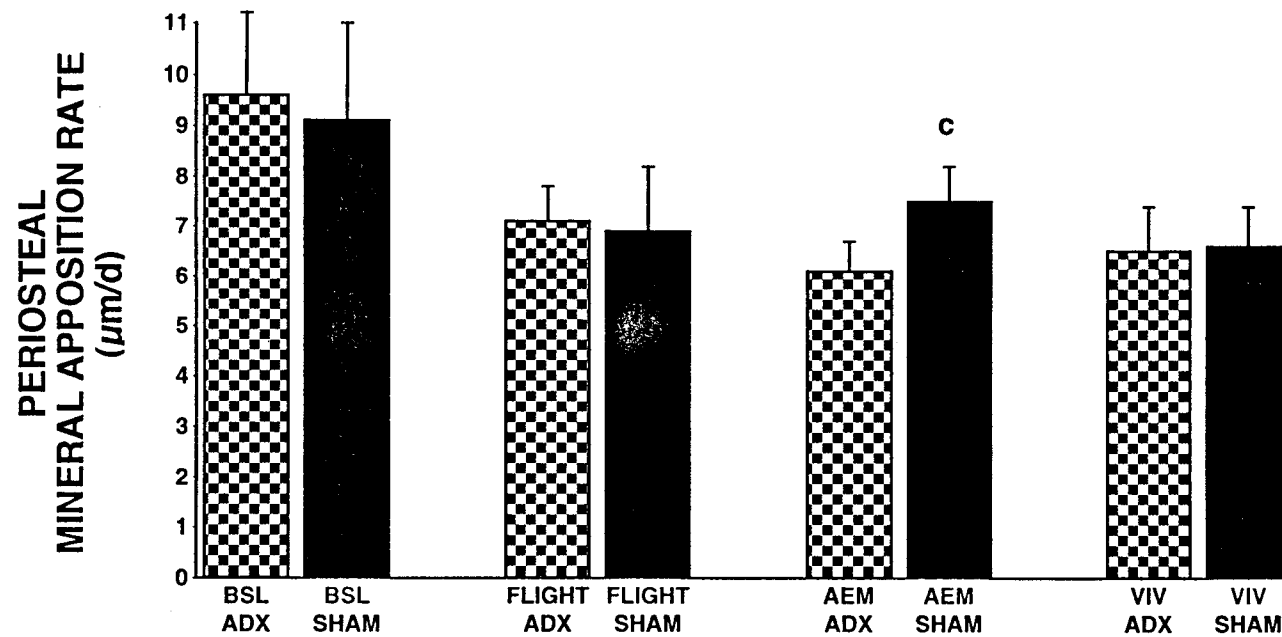
- a: vs Flight ADX ( $p < 0.05$ )
- b: vs Flight Sham ( $p < 0.05$ )
- c: vs AEM ADX ( $p < 0.05$ )
- d: vs AEM Sham ( $p < 0.05$ )
- e: vs VIV ADX ( $p < 0.05$ )

**FIGURE 10**



- a: vs Flight ADX ( $p < 0.05$ )
- b: vs Flight Sham ( $p < 0.05$ )
- c: vs AEM ADX ( $p < 0.05$ )
- d: vs AEM Sham ( $p < 0.05$ )
- e: vs VIV ADX ( $p < 0.05$ )

**FIGURE 11**



- a: vs Flight ADX ( $p < 0.05$ )
- b: vs Flight Sham ( $p < 0.05$ )
- c: vs AEM ADX ( $p < 0.05$ )
- d: vs AEM Sham ( $p < 0.05$ )
- e: vs VIV ADX ( $p < 0.05$ )

**FIGURE 12**